

Degeneration of DSS7 x D711 heterozygotes.

426

January 28, 1949.

Streaks out single colonies from Lac EMS of H-154-157.

8 colonies from each on Lac EMB

P30: Each shows Lac+ only! Known from initial
plates: Test 8 colonies from Lac EMS:

None of these show any signs of regeneration as Lac E143.

Conclude: H154-7 are not heterogeneous.

~~January 30, 1949.~~

427

January 30, 1949.

Y-161 7 sets. on lac E7413 (v). 30 plates ~~each~~ = 18,000
12 rechromed as lac-mutants (slow)

1/31/49.

W721 x Y410 100 tested all Lact++. Re-test #7+: Lact+

~~test for "++" in a spontaneous ultrazygote regard~~ 429.
W 553 C 102

Feb. 1, 1949.

~~td 721 x 440. m to t₄₅~~
W 477 x W 589. MEM Lac

20% Lac+ colonies streaked out. No clearly Lac₊.
Re streak 1-4 on Lac EMB for verification.

1A, B ++

2C, D are Lac₋; A+B are Lac++.

H173

3. ++

4. ++.

Streak out 4 cols. H173 in Lac EMB; Test 1+ and 1- and
test nutritional:

	BMTLB	BMT ₁ Ad	TLB, T ₁ Ad	Σ	V ₁	Lac
1A	+	-	+	+	S	+
1B	+	+	+	+	S	-
2A	+	+	+	+	S	-
2B	+	-	+	+	S	+
3A	+	+	+	+	S	-
3B	+	-	+	+	S	+
4A	+	+	+	+	S	-
4B	+	-	+	+	S	+

Growth in Σ rather sparse in 1A, 2B, 3B, 4B; Very heavy in
others.

Check from Σ tube on T1.

February 2, 1949

~~W₂₅₁~~ (Lac₃-S+) × W478 (Lac+ "H") on GluEMBS.

Majority are Glu+. High yield. Shoots out on E14B glucose.

100 tested on GluEMB. All Glu+

2/2/49.

Residual stock cultures of

blue

in EMB slns.

Lac

- 1 W2Y9
- 2 386 small cols. + slow +
- 3 387 compact
- 4 388 very thin.
- 5 389 slow +
- 6 423 minute cols
- 7 432 good -
- 8 433 " -
- 9 434 "
- 10 435 many + - good size.
- 11 467 good size -

in EMB Lac

check plates of W434 and W435, show papillae in their streaks with lytic clearings around them!! See 437-

check crosses: 2 plates each. xW108

- a. W433: 0+/400
- b. 435: 0+/150
- c. See 434. W467
- d W432 0/300
- e. 4434 No prototrophs. a few hundred microcolonies

Feb. 2, 1949.

~~Spored~~ S.O. W251 ($\text{lac}_3^{\text{Lac}} - \text{Sp}_3^+$) on EMB/ble to select for $\text{lac}_3^{\text{Lac}}$ reversions. Pick 8 papillae and streaks to purify + and - colonies noted generally. One colony was noted which looked  as if it might be segregating. *Intersayan?*

Pick from dark center and streaks as 432-1.:

Picks pure + from ~~the~~ remainder and streaks, for confirmation, on EMB-Mal.

432-1: mostly +. A few -. None could be identified as segregating (and this will be true except for the most stable intersayans).

Feb 1 ff. 1949.

Inoculate Y10 into T(m)T4B, Lac and Glc. Maintain loopful transfer
in homologous medium.

- B) A4.
- C) A5
- D) P6
- E) P8
- F) A9
- G) A10
- H) A11 → EMB selective
- I) A12 ←

etc.

February 6, 1979.

A. W589 x ~~W589~~ 466. 92 tested; Retest 5 for Lac^r.

B. W589 x 471 100 tested. No Lac^r!

A): 1-3 fairly certain Lac^r; 4-5?

1-3 yield approx. proportions
of Lac - prototrophic Cf. 429 where
several lac+ tested were prototrophic

1, 3 are Lac^r

H-174 and 175
(A) (B)

Test nutrition of Lac+ segregants:

A	1	+ in BM TLB, i.e. Ad T ₂ +
	2	"
	3	"
B	1	"

8 additional A 8 Ad T₂ + + ~~+~~?

8 " B. all Ad T₂ +

These stocks do not seem to be segregating nutr. req.

Suppressor tests

435

February 6, 1947.

W463

A. R21W12, +α Es.) x D461 .

B. W708 x "

B. 5 plates, ca 200/plate. No +. ∴ Lac₃-.

A. Picket and streak out on lactose

Feb. 5, 1949

Test Σ - segregants of H167 nutritionally to select for further "H" derivatives.

	W
1. TL	734
2. LB,	
3. TLB,	735
4. B,	
5. TL	736
6. TL	737
7. TL	738
8. TL	739
9. TL	740
10. TL	

Feb 6, 1949.

Dissolve 1/10 m 1/2 galactose + 1/2 glucose Pick Ga and Gc colonies to

- a) .1 ml 1/1000 OXPG + .2 ml KP buffer 1/10 pH 7.5
- b) .2 ml " "

After 3 hours incubation, Gla cells were - in both series; all but one was + from Gal series, 1 was rather weak. Strainsort
→ Justifying Method on LacE4B as #436-1.

Mixture of Lac+ and Lac-!

sp phage.

437

Feb 7 ff. 1949.

?

See 435. Stock cultures of W435 and w435, on Lac EMB, showed signs of plaque formation c a central papilla.

Picks uncontaminated colonies, and spread as detector, picks papillae and streaks out a) on W435^s (sensitive indicator) and -lac EMB for purification.

A 8.] Plaque formation clearly evident on 735^s.

Picks individual colonies from EMB and s.o., testing for phage also

A9: All 8 cultures carry considerable phage (comparable to number of bacteria in colloid streaks).

A9. Repeat

A10. Results is same sense. Conclude that these cultures are lysogenic on W435.

Stock, streaked, does not show this response.
w435

Filtered, many suspensions of # 437 "lysed area". Test on 410, W435:

Feb. 11, 1949.

1. NIH: prepare lysate in VSB from ~~active~~ plaques of 437-K or W435.

437 mature. | 5 further growth.

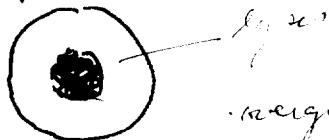
No action of control plaque noted on Y10, although active on W435.

2. Continue single colony isolations from 1 and 2 (lac + and - resp.) testing lysogenicity concurrently: 8 single colonies from 1 and 2 and 1 each from 3-8 were all lysogenic!

3. Test induction of 437-(4-8).

1-1: TLB, 1-2: TL(B,?) 2-1: poor? 2-2: do.

4. Typical appearance of a plaque is:



heavy regions of overgrowth,
occasional clear spots are seen, possibly
various mutants? ~~icks and scratches~~
~~438-~~ mass containing such clearings and spread: no
clearings noted!

5. Tests of Phage sensitivity.

	T ¹	T ²	T ³	T ⁴	T ⁵	T ⁶	T ⁷	C
Y10	S	S	R	S	S	S	S	R
438-1	S	S	R	S	S	S	S	R
W435	S	S	R	S	S	S	S	R or.

streaking.

∴ the lysogenic derivative has same phage reactions as the sensitive and standard strains.

Feb. 12, 1949

Look for lac⁺ among progeny of crosses of W167 segregants.

X 58-161

- A. W-736
 - B. W-737
 - C. W-738
 - D. W-740.
-

A.	356 tested.	No lac ⁺ .
B.	64 "	No lac ⁺
C.	100 "	No lac ⁺ .
D.	16 "	No lac ⁺

∴ Segregants of #167
do not carry Het.

report. mosquito: no age rule

440

Feb. 12, 1949.

w705 x w126. 15 plates. Ca. 50/plate = 750 colonies.

1, (mucoid), bac + found. 2.0. on EMD bac All mucoid.

spont. microsporidia: no sp. seen

440

Feb. 12, 1949.

w705 x w126. 15 plates. Ca. 50/plate = 150 colonies.

1, (mucoid), bac + found. 3.0 on EMB bac. All mucoid.

Feb. 12, 1949.

Harvest cells from EMR plate and suspend in 10 ml saline. Sediment and remove supernatant as λ . Filter most of the supernatant to remove bacteria. Keep a portion unfiltered, but substantially bacteria free. Wash sedimented cells with saline and resuspend.

- A) Dilute cells 10^3 and plate out: a) in EMR lac b) λ W435 for λ determination.
- B). Dilute λ ~~10^{-2}~~ 10^{-1} , 10^{-3} and 10^{-5} on 2 plate as above
11 plaques
- C) Titrate λ in W435.
- D) Heat 1:10 dilution of Cells A at 56°C . 1 hour. Titrate cells and phage
- A) (a) 50 colonies per plate on EMR Lac. Plaques very difficult to count
46, 38, 57 ~~25~~, 17, 34
- B) 10^{-5} 17 plaques; 7 bacterial colonies.
 10^{-1} Almost continuous lysis + overgrowing colonies.
~~10⁻³~~ Several hundred colonies; 40. plaques.
- C) No plaques at any dilutions.
- E) Test W753 as other coli strains:

34 single rolls. 58-161

and 35 of K-12 tested by short streak + .

purified on plate spread \pm w/35. Each camel +
Most readily scored on streaks. ~~=====~~

Cont'd

441g.

E. W435: Patchy lysis.

Y10 No plaque"

Y40 "

ML "

B11 "

B11,5 "

B14 "

W435 seems to be amorphous sensitive

Note: W753 is T-L-Lac₊-Glu₊₊.

W754 is (B)-M-Lac₃-.

Where W753 could have come from is not clear; possibly the original source of λ .

For further study, use W754 as a suitable strain.

Test R+S colonies from (B) above for lysogenicity on W435.

34 cols tested, all carried λ .

Transfer of λ .

442.

Feb. 13, 1949.

Moulate W754 in Y2 galactose with a series of other λ -2 types
to look for transfer of λ .

1. W754
2. 58-161
3. W-108
- 4 K-12

12 58-161 + W754
13 W108 "
14 K-12 "
15 W477 + "

On plates, test various cultures for sensitivity + content of λ .

Donor	W754	K-12	Y10
Host	w435	L	L?
		K-12	o
		w108	o
		w477	o

Conceivably, K-12 cannot λ and w435 is a mutation sensitive to it! See 443.

As important problem now is to devise best methods for scoring λ and obtaining it free from bacteria. (cover)

1. Show that λ is transmissible. Mix K-12 and W435 and streak out. Test Lac- colonies for λ , testing for its transfer.
2. Rapid methods for testing susceptibility + infection:
 - a. Try cross streaking
 - b. Spray developed colonies with suspension of λ .

February 15, 1949.

Test K-12, W753 + W754 for λ lysogeny in W435 stock + W753 old culture susc, in N5A. Also plate ca 150 K12 on W754 + mix with bacteri.

- 1) Plate: K-12 : no plaques; W754: 1 large plaque
- 2) Stake out: K-12: a few plaques noted in both sets of stakes. Latter more are found in 753 + 754. \therefore K-12 is lysogenic.
W435 is a susceptible mutant, and W753, etc. are mostly standards carrying λ .

Cross-streak tests for λ.

444.

Feb. 15, 1979

Laydown heavy streaks of W~~5~~754 (for λ) and 435 ($\mu_2\lambda^2$)
Cross-streaks in each other, K-12 cfc. on EMB lac

	NSA		EMB lac	
1. R	v. 754 λ ++	v. 435 λ ++	v. 754 ++	v. 435 +++
2. K-12	-	λ ++	-	++
3. SP-161	-	λ +	-	++
4. W478	? ±	±	-	++
5. W477	λ ++	++ ?	Patchy.	- +
6. W108	-	++	-	+
7. W595	-	++	-	+. Patchy appearance along entire streak

Cross-streaks are very difficult to read. However, ^{lac} λ or lac-S, on EMB lac are not so bad.

(N?) Lysate from penicillin treatment of K-12, 10^7 /ml initially, p'ttude.
2/17/79. 1 ml had no λ, was sterile.

Transfer of λ

445-

2/15/49.

Grow K12 with W435 for 8 hours in Y2 gal. Plate out on EMBlac.

$$(179+ : 8-) = \text{ca } 5\% - .$$

A) Pick + and test for λ on W435 EMBlac plates. Keep in order.

This test was inconclusive. Replica 16 and streakout on W435 film. 13 were apparently ~~sensitive~~ λ . 3, all of which had a lac+ component, showed λ . Pick + and - from each of these for retest. Pick others to test sensitivity. K12 controls had λ ; W435 did not. (445a)

#14. All + cols., $\lambda+$ and prototrophic. Not transfr.

#16. 2 - cols., $\lambda-$; lac+ was $\lambda+$. "

#11. 2 - " $\lambda-$; 2 + " " "

14 other - cols which were $\lambda-$ were tested and all found sensitive to λ .

B). W756, $\lambda+$ streaked out on W435. λ developed. Pick from confluent area to find lac-. Streak out on EMBlac.

Only 1 lac- colony found. Streak out $\frac{1}{5}$ W435 background. (not well isolated).

On lac EMBlac - Pure lac-; lac W435, lysogenic. Therefore transfer of λ can occur under these conditions. Reduced λ strain is W767 (check mutator). 4 colonies ill H-like W435. (See 445a).

C) Sensitivity tests in A were done with λ from K12. Streak out zones of lysis to find $\lambda+$ lac- for evidence of transfer. Do. (See 448).

Transf. of λ .
Recent + signed λ^+ stocks.

445a.

2/20/49.

B). All 4 s.c.i. of W767 agree in Glu-, M-, and λ^+ . All are pure from prep 1 as stock of induced lysogenicity.

C. Many plates were primarily Lac- with patchy plaques, and lysis at intersection of cutans; colonies. Pick Lac- colonies to test for λ^+ .

λ .	Autolys.	W	T	Autol.
a. 1-3 λ^+ 4 λ^-	1-3 - 4 +	432	+	-
b. 1 + 2 -	1 - 2 -	433	+	-
b' 1-2 +	1-2; -	434	-	-
c 2-3 + 1, 4 -	1-4; -	435	-	-
d. 1-4; +	-			
e 1-2; +	1+; 2-			
e' 1-2; +	1-2; -			
f 1-2 -	1-2 -			
f' 1-2 +	1+ 2 -			
g. +, +	-, -			
g' +, +	-, +			

W 434 + 435 are, therefore, merely λ^- . Their sensitivity was detected presumably as a result of mixture or contam. with W153, possibly related to W108.

Clear plaque noted. Pick as possible virus mutant and streak out on λ^- and λ^+

Autolysis probably indicates a sensitive strain which has phage mixed with it. In most cases, the autolysis and lysogenicity are comparable, consistent with this picture.

UV - Lac Mutationism.

446

2/16/49.

- 1) W760 43 pl x ca 50 / 2500 colonies. Very high yield of mutants apparent.
- 2) W758. 40 pl. ca 50 / 2000 cols.

1) 5-- w768-772

2 ± w773-774

3 slow w775-777.

2). 5- w778-782.

w	bac	Mal	Glu	Gra	Gal
768	-	-	-	-	-
9	-	+	+	+	+
770	-	-	-	+	±
1	-	+	+	+	+
2	-	+	+	+	+
3	±	±	±	+	+
4	±	±	+	+	+
5	±	±	+	+	+
6	±	±	+	+	+
7	±	±	+	+	+
8	±	±	+	+	+
9	±	±	+	+	-
780	±	±	+	+	+
1	-	-	-	-	+
2	-	-	-	-	+

Hex -
 bac -
 (108)
 Lac
 Lac
 (108')
 (108)
 slowbac
 slowbac
 "
 Lac
 Lac
 Gal - slowbac
 (108'?)
 Lac

2/18/49.

Stockout W467 on EM13 lactose. Restreak, and pick lac+ colonies to EM13 Mal + Glu.

27 tested on Glu + Mal. 3 Glu - Lac+. All others +. Water, 33 test on Mal. All +.

Purify the Glu-lact+ on EM13 Lac. Allure Mal - W764-766.

Resuscitate W766 on Glu, EM13 to recover resuscensis.

2/21/49. Resuscitate W768 on Glu, etc. media tried specific resuscensis.
Maltose: slow+. Lactose full+. Nothing on gal, MtL or glucose

2/23. Collect W677 lac+. Check on other sugars. W814

2/23/1. Test 1 Mal+, 8 lac+ purified from homologous plates.

	Glu	Mal	MtL	Gal	Lac	
1	-	+	++	-	-	+ + W815
2	-	+	++	-	-	++
3	-	+	++	-	-	++
4	-	-	-	-	-	± + W816
5	-	+	++	-	-	++
6	-	+	++	-	-	++
7	-	+	++	-	-	++ W817
PZVH						

not spec. Lac response! Save 1, 4, 7. as W815-817

446a.

3/9/49.

Type. A set of "Hali" colonies was tested on 4 sugars. Many undoubtedly subs.

Hal Lac Gal Glu W

1 + - - 856

2 + ++ ++ 857

3 ++ ↓ 858

3/2/49.

5 Gal + isolated and tested:

	Gal	Lac	Glu	Mal	Mtl	
1	+	+	=	+	-	840
2	+	+	=	+	-	
3	+	+	=	+	-	
4	+	+	=	+	-	
5	+	+	-	+	-	8-7

Fermentation of Gal is sluggish; Mal and Lac slow.
 Pick as (W) 839 + 840

Routine tests for λ .

447

2/18/49.

Preliminary tests have shown λ is K-12 and a number of derivatives. Re-test + check by streaking out on EMBS sugar, and on W435.

	λ	On W435.	Autolysis.
1. K-12	+	-	-
2. W754	+	-	-
3. 58-161	+	-	-
4. Y40	+	-	-
5. Y87	+	-	-
6. Y70	+	-	-
7. W677	+	-	-
8. W70 71	+	-	-
9. W45	+	-	-
10. Y10	+	-	-
11. Y55 477	+	-	-
12. Y11 W754	+	-	-
13. W 55 125	+	-	-
14. W680	+	-	-
15. W677 125	+	-	-
16. W478	+	-	-
17. W467	+	-	-
18. W108	+	-	-
19. W145	+	-	-
20. W126	+	-	-

21

∴ Most standard stocks still carry λ and are resistant to it.

2/18/49.

EML noted that W518 A + B were lysed by Y10. W518 itself, when streaked out was autolytic, suggesting a mixture of λ^- and λ^+ .

1. Streak out W518 on EMB Lac

2. Test A + B for lysis of each other, of W435, and by K-12.

Can. \rightarrow A B W435 K12
Host ↓

W435	λ^-	λ^-	λ^-	λ^+
A	-	-	-	+
B	-	-	-	+

\therefore 518A + B show same pattern of sensitivity as W435 and are λ^+, λ^-

Test for transfer of λ from K-12 to 518A + B. Stake out plaques to find λ^+ Lac- I. C is λ / 518.

Mostly + colonies. Some - had plaques. Pick clear lac- colonies + streak out as EMB Lac; EMP W435.

A). ~~1-4~~ 1-4 λ^+ (3 had 1 plaque), 1; 3 are autolytic. Use (2).

B. 1-4 all λ^+ no autolysis. 3 has papillae, probably not pure -.
Use #1.

C.) (W518 λ^+). $\begin{matrix} 1 & - & \text{W435} \\ 2 & - & \text{(1 pl.)} \\ 3 & - & \text{no growth} \\ 4 & ++ & - \end{matrix} \rightarrow \rightarrow \rightarrow$ W518 λ^+

$\lambda^- \times \lambda^+$.

449

2/18/49.

Y10 x W435

8 colonies found in 15 plates. Very low yield! All best
 streak out on Lact TYB. Use 2 ~~colonies~~ colonies per plate, to give
 $(A-D)(1-4)$.

Retest D1:

1/435 auto.			D1/518 No plaques.			
A	1	+	-	Y10/D1 Some questionable plaques.	Y10/518 Numerous plaques & central papilla	
	2	+	-			
	3	+	-			
	4	+	-			
B	1	+	-	D1.		
	2	+	-	D1.		
	3	+	-	D1.		
	4	+	-	D1.		
C	1	+	-	D1.		
	2	+	-	D1.		
	3	+	-	D1.		
	4	+	-	D1.		
D	1	-?	-	Retest: was sensitive to λ .		
	2	+	-	Retests 4/7/49.		
	3	+	-	Sensitive to λ . λ^-		
	4	+	-	Sensitive to λ . λ^-		

2/23/49. Test ~~19~~¹⁹ segregants from 10 mosaics of λ^{176} ($w518 \times w5788$)

1/518 auto.			1/518 auto.			1/518 auto.			
A	1	+	-	B	+	-	C	+	-
	2	+	-		+	-		+	-
	3	+	-		+	-		+	-
	4	+	-		+	-		+	-
D	1	+	-	E	+	-			
	2	+	-		+	-			
	3	+	-		+	-			
	4	+	-		+	-			

Attempts to obtain free λ

450.

2/19/49.

- A. Scrape area of lysis of W758/W435 into H₂O. sediment and filter supernatant (without glass).
- B. Extract 100 mg dried K-12 in 10 ml H₂O. Sediment 1:10 dil. and filter supernatant. B' is test sediment. 9 colonies K12/1ml noted. Numerous tiny cont.
^(fewer than?)
- C. Inoculate Y2 in W435 and K-12, young cultures. Shake + incubate ca 2-3 hrs. Sediment and filter.
- c' Let grow overnight and filter.

A). .1ml: ~~ca~~ 80 plagues on ~~W758~~ W435 on EM 13-S. ✓
A loopful streaked out was similarly effective.

B. No plaques.

B' 2 plaques in loopful, probably from K-12.

C. No plaques in .1ml. 1/plate; 2 plaques on another.

Free phage from A) only.

C': ca 500 plaques / .1ml i.e. titer of ca 5000.

Synergism in lac tests
 Lac₁, ..., Lac₇.

450

2/19/49.

1.
 w112

2. w45

3. w108

4. w126

5. w145

6. w125

7. w133.

Cross streak on EMB lac.

	1	2	3	4	5	6	7
1	-	-	-	-	-	++	-
2	-	-	-	-	-	++	-
3	-	-	-	-	-	-	-
4	-	-	-	-	-	+	-
5	-	-	-	-	-	+	-
6	++	++	++	++	++	±	++
7	-	-	-	-	-	++	-

At 24 hours, Lac₆ - reacted regularly. Its isolated response was irregular, sometimes ++, sometimes -! Needs study in liquid medium!

Held for 48 hr. No change Lac₆ shows most interesting interactions

Segregation of M179.

460

2/26/49.

M179 is W126 x W778. (T_{LB}, Lac₊ x IV. Hist Lac, -).

Streak out original var. cols. on Lac EMB. Practically no pure +. Purify 1 - from each. Pick + entries for new segregants. Test mutation of 6 additional - segregants from different bac. Also streak out 16 additional v colonies.

a --
b growth - H only.
c ++ (weak on - T)
d --
e --
f ++
g --
h --
i --
j TL

unstreaked diff. to establish. Probably IV requirement interferes.